PATENT
Docket No. 226272001403

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Tatania Grollman

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

James M. ALLEN

Serial No.: To Be Assigned

Filing Date: Herewith

For: PACKAGING CELL LINES FOR GENERATION OF HIGH TITERS OF RECOMBINANT AAV VECTORS

Examiner: To Be Assigned

Group Art Unit: To Be Assigned

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to examination, please amend the above-referenced application as follows:

pa-544676

AMENDMENTS

In the Specification

On page 1, after the title of the invention, please insert the following paragraph:

--CROSS-REFERENCE TO RELATED APPLICATIONS

-- This application is a continuation of U.S. Patent Application Serial No. 08/564,167, filed December 14, 1995, which is the U.S. National Phase of International Application PCT/US95/15892, filed on December 6, 1995, which claims priority to U.S. patent application 08/480,575, which was filed June 7, 1995, now abandoned, and to U.S. patent application 08/350,219, which was filed December 6, 1994, now abandoned. --

In the claims:

Please cancel claims 18-20.

Please amend claims 1, 3, 6-12, 15-17 and 21 as follows:

1. (Amended) A method of producing a mammalian cell [capable of high efficiency] for packaging of a recombinant AAV (rAAV) vector, said method comprising the steps of:

(a) providing a mammalian cell which comprises a stably integrated AAV cap gene operably linked to a promoter, and a stably integrated AAV rep gene operably linked to a helper virus-inducible heterologous promoter, wherein p5 promoter function has been replaced by the helper virus-inducible heterologous promoter;

(b) replicating the cell of step (a) to produce a population of cells; and

(c) introducing a helper virus to the population of cells of step (b); [and

(d) selecting a cell exhibiting helper-virus-inducible rep protein activity.] ,wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

3. (Amended) A method according to claim 1, wherein said packaging cell [is capable of growing] grows at least one half as rapidly as parental-type cells that do not contain an AAV rep gene, and wherein said packaging cell when used to package rAAV vectors [is capable of packaging rAAV vectors to produce] produces at least 100 rAAV particles/cell.

6. (Amended) A cell produced by the method of claim 1, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

7. (Amended) A cell produced by the method of claim 3, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

8. (Amended) A cell produced by the method of claim 4, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

9. (Amended) A cell produced by the method of claim 5, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

10. (Amended) A mammalian cell [capable of high efficiency] for packaging of a recombinant AAV (rAAV) vector, said cell comprising a stably integrated cap gene operably linked to a promoter, and a stably integrated rep gene operably linked to a helper virus-inducible heterologous promoter; wherein p5 promoter function has been replaced by the helper virus-inducible heterologous promoter and wherein said cell exhibits helper-virus-inducible [rep protein activity] expression of said stably integrated AAV rep gene.

11. (Amended) An AAV packaging cell of claim 10, wherein said helper-virus-inducible [rep protein activity] expression of said stably integrated AAV rep gene is inducible by adenovirus.

12. (Amended) An AAV packaging cell of claim 10, wherein said packaging cell [is capable of growing] grows at least one half as rapidly as parental-type cells that do not contain an AAV rep gene, and wherein said packaging cell when used to package rAAV vectors [is capable of packaging rAAV vectors to produce] produces at least 100 rAAV particles/cell.

15. (Amended) An AAV packaging cell of claim 10, further comprising a stably integrated recombinant AAV (rAAV) vector, said vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter.

16. (Amended) A method of packaging a recombinant AAV vector, comprising the steps of:

(a) providing an AAV packaging cell of claim 10;

(b) introducing a recombinant AAV (rAAV) vector, said vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter;

(c) introducing a helper virus; and

(d) incubating the cell under conditions suitable for replication and packaging of AAV such that said rAAV vector is packaged.

17. (Amended) A method of packaging a recombinant AAV vector, comprising the steps of:

(a) providing an AAV packaging cell of claim 15 which comprises a stably integrated rAAV vector comprising a polynucleotide of interest operably linked to a promoter;

(b) introducing a helper virus; and

(c) incubating the cell under conditions suitable for replication and packaging of AAV such that said rAAV vector is packaged.

21. (Amended) A method of determining the [relative] infectious titer of an rAAV vector preparation, comprising the steps of:

(a) introducing a helper virus and serial dilutions of the rAAV vector preparation to AAV packaging cells of claim 10;

(b) incubating the cells under conditions suitable for replication of AAV; and

determining the amount of replicated rAAV vector relative to an rAAV preparation of known titer.

Please add new claim 22 as follows:

22. (New) The method of claim 1, further comprising the step of selecting a cell exhibiting helper-virus-inducible expression of said stably integrated AAV rep gene.

REMARKS

Claims 18-20 have been cancelled, and claims 1, 3, 6-12, 15-17 and 21 have been amended. New claim 22 has been added. Support for the amendments is found in the specification, inter alia, at page 26, lines 12-13 and page 23, lines 4-8. A copy of the claims as amended is provided for the Examiner's convenience in the enclosed Appendix A. Please substitute the amended claim set.

With respect to all amendments and cancelled claims, Applicants have not dedicated or abandoned any unclaimed subject matter and moreover have not acquiesced to any objection and/or rejection made by the Office. Applicants expressly reserve the right to pursue prosecution of any presently excluded subject matter or embodiments in one or more future continuation and/or divisional applications.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 226272001403. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

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Appendix A

Pending Claims 22627-20014.03

1. (Amended) A method of producing a mammalian cell for packaging of a recombinant AAV (rAAV) vector, said method comprising the steps of:

(a) providing a mammalian cell which comprises a stably integrated AAV cap gene operably linked to a promoter, and a stably integrated AAV rep gene operably linked to a helper virus-inducible heterologous promoter, wherein p5 promoter function has been replaced by the helper virus-inducible heterologous promoter;

(b) replicating the cell of step (a) to produce a population of cells; and

(c) introducing a helper virus to the population of cells of step (b);

(d) wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

2. A method according to claim 1, wherein said helper virus is an adenovirus.

3. (Amended) A method according to claim 1, wherein said packaging cell grows at least one half as rapidly as parental-type cells that do not contain an AAV rep gene, and wherein said packaging cell when used to package rAAV vectors produces at least 100 rAAV particles/cell.

4. A method according to claim 1, wherein said mammalian cell of step (a) comprises the combined rep and cap genes of AAV in which the p5 promoter has been replaced by a heterologous promoter.

5. A method according to claim 4, wherein said heterologous promoter is a mouse metallothionein I (mMT-I) promoter.

6. (Amended) A cell produced by the method of claim 1, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

7. (Amended) A cell produced by the method of claim 3, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

8. (Amended) A cell produced by the method of claim 4, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

9. (Amended) A cell produced by the method of claim 5, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

10. (Amended) A mammalian cell for packaging of a recombinant AAV (rAAV) vector, said cell comprising a stably integrated cap gene operably linked to a promoter, and a stably integrated rep gene operably linked to a helper virus-inducible heterologous promoter; wherein p5 promoter function has been replaced by the helper virus-inducible heterologous promoter and wherein said cell exhibits helper-virus-inducible expression of said stably integrated AAV rep gene.

11. (Amended) An AAV packaging cell of claim 10, wherein said helper-virus-inducible expression of said stably integrated AAV rep gene is inducible by adenovirus.

12. (Amended) An AAV packaging cell of claim 10, wherein said packaging cell grows at least one half as rapidly as parental-type cells that do not contain an AAV rep gene, and wherein said packaging cell when used to package rAAV vectors produces at least 100 rAAV particles/cell.

13. An AAV packaging cell of claim 10, wherein said cell comprises the combined rep and cap genes of AAV in which the p5 promoter has been replaced by a heterologous promoter.

14. An AAV packaging cell of claim 13, wherein said heterologous promoter is a mouse metallothionein I (mMT-I) promoter.

15. (Amended) An AAV packaging cell of claim 10, further comprising a stably integrated recombinant AAV (rAAV) vector, said vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter.

16. (Amended) A method of packaging a recombinant AAV vector, comprising the steps of:

(a) providing an AAV packaging cell of claim 10;

(b) introducing a recombinant AAV (rAAV) vector, said vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter;

(c) introducing a helper virus; and

(d) incubating the cell under conditions suitable for replication and packaging of AAV such that said rAAV vector is packaged.

17. (Amended) A method of packaging a recombinant AAV vector, comprising the steps of:

(a) providing an AAV packaging cell of claim 15 which comprises a stably integrated rAAV vector comprising a polynucleotide of interest operably linked to a promoter;

(b) introducing a helper virus; and

(c) incubating the cell under conditions suitable for replication and packaging of AAV such that said rAAV vector is packaged.

21. (Amended) A method of determining the infectious titer of an rAAV vector preparation, comprising the steps of:

(a) introducing a helper virus and serial dilutions of the rAAV vector preparation to AAV packaging cells of claim 10;

(b) incubating the cells under conditions suitable for replication of AAV; and

determining the amount of replicated rAAV vector relative to an rAAV preparation of known titer.

22. (New) The method of claim 1, further comprising the step of selecting a cell exhibiting helper-virus-inducible expression of said stably integrated AAV rep gene.